PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

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Date:	26 JUL 2004
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PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Rule 71.1)

Date of mailing *(day/month/year)*	23.07.2004
Applicant's or agent's file reference: P32590WO/NCB	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB 03/03192	25.07.2003	26.07.2002

Applicant: ROSLIN INSTITUTE (EDINBURGH) et al.

DOCKETING
NOTED

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

The applicant's attention is drawn to Article 33(5), which provides that the criteria of novelty, inventive step and industrial applicability described in Article 33(2) to (4) merely serve the purposes of international preliminary examination and that "any Contracting State may apply additional or different criteria for the purposes of deciding whether, in that State, the claimed inventions is patentable or not" (see also Article 27(5)). Such additional criteria may relate, for example, to exemptions from patentability, requirements for enabling disclosure, clarity and support for the claims.

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PATENT COOPERATION TREATY

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

26 JAN 2005

Applicant's or agent's file reference P32590WO/NCB	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/GB 03/03192	International filing date *(day/month/year)* 25.07.2003	Priority date *(day/month/year)* 26.07.2002
International Patent Classification (IPC) or both national classification and IPC C12N15/62		
Applicant ROSLIN INSTITUTE (EDINBURGH) et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 4 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
26.02.2004	23.07.2004
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer van Heusden, M Telephone No. +49 89 2399-8145

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB 03/03192

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-49 as originally filed

Claims, Numbers

1-15 received on 08.07.2004 with letter of 07.07.2004

Drawings, Sheets

1/21-21/21 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☐ contained in the international application in written form.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority in written form.

   ☐ furnished subsequently to this Authority in computer readable form.

   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-15
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-15
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	
	No:	Claims	1-15 (?)

2. Citations and explanations

   see separate sheet

Additional remarks to section V:

1. Novelty (Article 33(2) PCT)

1.1 The present application discloses the use of a nucleic acid construct, comprising a sequence encoding a lipocalin, for the detection of a gene activation event resulting from a change in a cell. Said change may be toxicological stress, a metabolic change or a disease.

1.2 The documents mentioned in this communication are numbered as in the International Search Report (ISR), i.e. D1 corresponds to the first document of the ISR etc.

1.3 The present application satisfies the criterion set forth in Article 33(2) PCT because the subject matter of claims 1-15 is novel in view of the cited documents.

2. Inventive step (Article 33(3) PCT)

It seems that none of the cited prior art documents discloses the use of a member of the lipocalin protein family as a reporter gene. Lipocalins are know in the art, their fusion with epitopes is known (e.g. in D1 or D7) but none of the documents suggests their usefulness as a reporter gene. Therefore it seems that the use of a lipocalin as a reporter gene, or a method of detecting gene activation using a lipocalin reporter construct, can be considered inventive (claims 1-15).

3. Industrial applicability (Article 33(4) PCT)

The subject matter of claims 1-11 relates to a use for detection of a gene activation event in vitro or in vivo. Said gene activation event can be a disease. Thus the claims encompass a method of diagnosis performed in vivo on the human or animal body. The subject matter of claims 12-15 encompasses a method of diagnosis of a disease performed on a non-human animal. Thus the subject matter of claims 1-15 includes methods of diagnosis of the human or animal body and is thus excluded from examination by Article 34(4)(a)(I) PCT in combination with Rule 67(iv) PCT. For the assessment of these claims on the question whether they are industrially applicable, no unified criteria exist in PCT. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject matter

of claims to the use of a compound in medical treatment, but will allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment. The applicant is already informed that in the case of a European application, claims 1-15 do not seem to be allowable because 'diagnostic methods practised on the human or animal body shall not be regarded as inventions which are susceptible of industrial application'.

CLAIMS

1. The use of a nucleic acid construct comprising a nucleic acid sequence encoding a member of the lipocalin protein family as a reporter gene for the detection of a gene activation event resulting from a change in or altered metabolic status in a cell *in vitro* or *in vivo*, wherein said cell is transfected with said construct.

2. The use as claimed in claim 1, in which the lipocalin protein is heterologous to the cell in which it is expressed.

3. A use as claimed in claim 1, in which the lipocalin protein is coded for by a nucleic acid construct comprising (i) a nucleic acid sequence encoding a member of the lipocalin protein family, and (ii) a nucleic acid sequence encoding a peptide sequence of from 5 to 250 amino acid residues

4. A use as claimed any one of claims 1 to 3, in which the lipocalin is selected from the group consisting of: ovine betalactoglobulin (BLG) (accession No. X12817), murine major urinary protein (MUP) (accession No. NM 031188) and rat α-2-urinary globulin (α-2u) (accession number M27434).

5. A use as claimed in claim 3 or claim 4, in which peptide sequence is an epitope.

6. A use as claimed in claim 5, in which the epitope is selected from the group consisting of EQKLISEEDL, GKPIPNPLLGLDST, YPYDVPDYA, NVRFSTIVRRRA, KQMSDRRENDMSPS, SGNEVSRAVLLPQSC, SSLSYTNPAVAATSANL, RSTLQHPDYLQEYST, VSTLLRWERFPGHRQA, KFQQLVQCLTEFHAALGAYV, QEQCQEVWRKRVISAFLKSP, and RLSDKTGPVAQEKS

7. A use as claimed in any one of claims 2 to 6, in which the construct additionally comprises a promoter element upstream of the (i) a nucleic acid sequence encoding a member of the lipocalin protein family, and (ii) and nucleic acid sequence encoding a peptide sequence of from 5 to 250 amino acid residues.

8. A use as claimed in claim 7, in which the promoter element may be selected from one of the following groups consisting of :

(i) c-myc, p21/WAF-1, MDM2, Gadd45, FasL, GAHSP40, TRAIL-R2/DR5, BTG2/PC3;

(ii) MnSOD, CuZnSOD, IκB, ATF4, xanthine oxidase, COX2, iNOS, Ets-2, FasL/CD95L, γGCS, ORP150.

(iii) Lrg-21, SOCS-2, SOCS-3, PAI-1, GBP28/adiponectin, α-1 acid glycoprotein, metallothioneine I, metallothioneine II, ATF3, IGFbp-3, VDGF and HIF1α.

(iv) Gadd 34, GAHSP40, TRAIL-R2/DR5, c-fos, CHOP/Gadd153, APAF-1, Gadd45, BTG2/PC3, Peg3/Pwl, Siah1a, S29 ribosomal protein, FasL/CD95L, tissue tranglutaminase, GRP78, Nur77/NGFI-B, CyclophilinD, p73 and Bak.

(v) a promoter from a xenobiotic metabolising cytochrome p450 enzymes from the 2A, 2B, 2C, 2D, 2E, 2S, 3A, 4A and 4B gene families.

(vi) a synthetic promoter sequence comprised of a minimal eukaryote consensus promoter operatively linked to one or more response elements selected from the group consisting of the aryl hydrocarbon (Ah)/Ah nuclear translocator (ARNT) receptor response element, the antioxidant response element (ARE), the xenobiotic response element (XRE).

9. A use as claimed in claim 1, in which the nucleic acid construct comprises a stress inducible promoter operatively isolated from a nucleic acid sequence encoding a member of the lipocalin protein family by a nucleotide sequence flanked by nucleic acid sequences recognised by a site specific recombinase, or by insertion such that it is inverted with respect to the transcription unit encoding a member of the lipocalin protein family, in which the construct additionally comprises a nucleic acid sequence comprising a tissue specific promoter operatively linked to a gene encoding the coding sequence for the site specific recombinase.

10. A use as claimed in claim 9, in which the site specific recombinase sequences are two *lox*P sites of bacteriophage P1.

11. A use as claimed in any one of claims 1 to 10, in which the gene activation event is induction of toxicological stress, metabolic changes, or disease, including a disease that is the result of viral, bacterial, fungal or parasitic infection.

12. A method of detecting a gene activation event in a cell *in vitro* or *in vivo*, comprising assaying a host cell stably transfected with a nucleic acid construct comprising a nucleic acid sequence encoding a member of the lipocalin protein family, or a transgenic non-human animal whose cells express such a construct, in which the cell or animal is subjected to a gene activation event that is signalled by expression of a peptide tagged lipocalin reporter gene.

13. A method of screening for, or monitoring of toxicologically induced stress in a cell or a cell line or a non-human animal, comprising the use of a cell, cell line or non human animal which has been transfected with or carries a nucleic acid construct as defined in any one of claims 2 to 10.

14. A method for screening and characterising viral, bacterial, fungal, and parasitic infection comprising the use of a cell, cell line or non human animal which has been

transfected with or carries a nucleic acid construct as defined any one of claims 2 to 10.

15. A method for screening for cancer, inflammatory disease, cardiovascular disease, metabolic disease, neurological disease and disease with a genetic basis comprising the use of a cell, cell line or non human animal which has been transfected with or carries a nucleic acid construct as defined in any one of claims 2 to 10.